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Incidence of JC Viruria Is Higher Than That of BK Viruria in Taiwan

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To investigate the prevalence of human polyomaviruses in Taiwan, urine samples from immunocompetent (healthy), transient immunocompromised (pregnant), and prolonged immunosuppressed (autoimmune disease) individuals were collected throughout the island. The viral DNA in the urine was detected by the polymerase chain reaction (PCR) and Southern blot. The viral genotypes were determined by DNA sequencing within the regulatory region. The overall results, including cases reported previously, show that 13.3% (10/75) of immunocompetent individuals, 26.0% (20/77) of pregnant women, and 37.5% (18/48) of autoimmune disease patients are JCV positive. All of the immunocompetent individuals are BKV negative, but 3.9% (3/77) of the pregnant women and 6.2% (3/48) of autoimmune disease patients are BKV positive. Twenty-four percent (48/200) of the examined urine samples were JCV positive, but only 3% (6/200) were BKV positive. JCV positive individuals were mainly infected with CY (42%) and TW-1 (52%) subtypes. These results suggest that the incidence of urinary excretion of human polyomaviruses in immunosuppressed individuals is higher than that of immunocompetent individuals. The prevalence of JCV appears to be higher than that of BKV in Taiwan. In addition, CY and TW-1 are the predominant subtypes of JCV prevalent in the Taiwanese population. ***J. Med. Virol. 52:253–257, 1997.***

KEY WORDS: papovaviruses; human polyomavirus; viral prevalence

INTRODUCTION

The human polyomaviruses, JC virus (JCV) and BK virus (BKV), infect human populations all over the world [Walker and Frisque, 1986]. Primary infection occurs in childhood, and by adult age, serum antibodies can be detected in 70 to 80% of individuals [Padgett and Walker, 1973; Brown et al., 1975; Taguchi et al., 1982; Chesters et al., 1983]. Although it is not clear whether both viruses are associated with any disease in the general population, JCV and BKV have been linked to progressive multifocal leukoencephalopathy [Padgett and Walker, 1973; Padgett et al., 1976, 1977] and hemorrhagic cystitis [Arthur et al., 1986; Apperley et al., 1987] respectively, particularly in immunocompromised patients. The viruses persist latently in renal tissue after infection [Yogo et al., 1990], and may be reactivated and shed in the urine during pregnancy or as a state of immunodeficiency such as in patients with AIDS, individuals receiving leukemia therapy, or transplant recipients [Chesters et al., 1983; Arthur et al., 1986; Apperley et al., 1987]. JC and/or BKV viruria have been reported in 3% of pregnant women [Coleman et al., 1980], and in 10 to 65% of patients from various groups with an immunosuppressed state [Hogan et al., 1980; Gardner et al., 1984; Walker and Frisque, 1986].

Previously, four different subtypes were found in Taiwan: CY, TW (Taiwan)-1, TW-2, and TW-3 of JCV and two archetypal strains, TC (Taichung)-1 and TC-2, of BKV in pregnant women [Chang et al., 1996a] and patients with autoimmune diseases [Chang et al., 1996b]. CY archetype of JCV was first isolated from the urine samples of nonimmunocompromised individuals [Yogo et al., 1990]. TW-1 JCV has a copy of pentanucleotide (GGGAA) deletion at nucleotide 218–222; TW-2 has two identical copies of pentanucleotide (GG-

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GAA) deletions at nucleotide 198-202 and 218-222; TW-3 has two different pentanucleotide (AAAGC and GGGAA) deletions at nucleotide 188–192 and 218–222 within the regulatory region when compared to CY JCV. TC-1 and TC-2 BKV have nucleotide alterations within the regulatory region when compared to WW archetype of BKV [Rubinstein et al., 1987]. The previously examined pregnant women [Chang et al., 1996a] and autoimmune disease patients [Chang et al., 1996b] resided mainly in the west area (Taichung City) of Taiwan. In order to identify which genotypes of JCV and BKV prevail in Taiwan, investigation was extended to collect additional urine samples from 75 healthy individuals, 46 pregnant women and 28 autoimmune disease patients around the Island of Taiwan. In this study, it was found that CY and TW-1 strains of JCV are predominant in Taiwan area but BKV does not appear to be highly prevalent in Taiwan.

MATERIALS AND METHODS

Collection of Urine Samples

One hundred forty-nine individuals were enrolled in the current study and divided into three groups. Group I includes 75 immunocompetent students at Chung Shan Medical and Dental College between the ages of 20 to 26 from across Taiwan. Eight of them were from the east, 24 from the west, 14 from the south, and 29 from northern Taiwan. Group II includes 46 newly registered pregnant women; 15 from the east, 16 from the west, and 15 from northern Taiwan. The urine samples were collected during the second trimester, 16 to 18 weeks of pregnancy, and from different obstetrics and gynecology clinics. Group III includes 28 newly registered autoimmune disease patients receiving immunosuppression treatment at the Hospital of Chung Shan Medical and Dental College. Among them, 14 patients with rheumatoid arthritis (RA) were diagnosed according to the 1987 revised American Rheumatism Association (ARA) criteria for RA [Arnett, 1988]; nine patients with systemic lupus erythemaosus (SLE) according to the 1982 revised criteria of ARA for the classification of SLE [Tan et al., 1982]; three patients with dermatomyositis/polymyositis (DM/PM) according to the diagnostic criteria of Bohan and Peter [1975]. One patient with Sjogren's syndrome (SS) was confirmed by a history of dry eye and mouth, positive Shirmer's test, corneal punctate erosions, and lip biopsy. One patient with adult Still fulfilled the criteria of Medsger and Christy [1976]. All of the urine specimens were frozen immediately at −20°C after collection until tested.

Methods

The detailed protocols for sample preparation, PCR amplification, Southern blotting, and DNA sequencing were described previously [Chang et al., 1996a,b]. These procedures are briefly described below.

Sample Preparation

Ten milliliters of urine were ultracentrifuged at 142,000*g* × 90 min. The pellet was resuspended in 1 ml of distilled water by brief vortexing. Five μl of the resuspended fluid was mixed with 4 μl of water and 1 μl of 10 X lysis buffer containing proteinase K (100 mM Tris-HCl, 10 mM EDTA, pH 8.0, and 500 μg/ml proteinase K). The mixture was incubated at 50°C for 15 min and then at 95°C for 10 min, and centrifuged at 10,000 rpm for 3 min. The supernatant was collected and used for a polymerase chain reaction.

Polymerase Chain Reaction (PCR)

The polymerase chain reaction was carried out for 40 cycles in a total volume of 50 μl. The primers applied were JBR1 (5′-CCTCCACGCCCTTACTACTTCTGAG-3′) and JBR2 (5′-GTGACAGCTGGCGAAGAAC-CATGGC-3′) [White et al., 1992] which anneal to the constant ends of regulatory regions (nucleotide −45 to −21 and 265 to 289) of both JCV and BKV.

PCR Products Analysis by Electrophoresis

After amplification, 5 μl of the PCR reaction mixture was analyzed by electrophoresis in a 1.5% agarose gel in a TBE buffer (90 mM Tris-HCl, pH 8.3, 90 mM sodium borate, 2.5 mM EDTA) at 80 volts for 30 min. The agarose gel was stained with ethidium bromide (0.5 μg/ml) and photographed under UV light.

Southern Blot of PCR Product

Ten μl of the PCR reaction mixture were electrophoresed in a 1.5% agarose gel in a TAE buffer (40 mM Tris-acetate, pH 8.5, 2 mM EDTA). The DNA fragments in the agarose gel were transferred onto nylon membrane (MSI, MA, USA) by using semi-dry Horizblot (ATTO Corp., Tokyo, Japan) at 3 mA per cm^2 of nylon membrane for 3 hr. The detail protocols were provided by the company. Briefly, the membrane was treated with an alkaline solution (0.2 N NaOH and 0.6 M NaCl) for 1 hr and then with Tris buffer (1.0 M Tris, pH 7.4 and 0.6 M NaCl) for 1 hr. Prehybridization was followed by treating the membrane with 5 × Denhardt's solution and 6 × SSC (0.9 M NaCl and 0.09 M sodium citrate) at 58°C for 5 hr. The solution was replaced with a fresh hybridization solution of 5 × Denhardt's, 6 × SSC, and 0.5% sodium dodecyl sulfate (SDS) containing 4×10^5 cpm/ml of (^{32}P) ATP-end-labeled JBR-3 oligonucleotide probe (5′-TGGCAGTTATAGTGAAAC-CCC-3′). JBR-3 oligonucleotide probe annealed at nucleotide 140–160 for JCV and 117–138 for BKV regulatory regions. Following 5 hr of hybridization at 58°C with shaking, the nylon membrane was washed five times with 2 × SSC solution with 1.0% SDS at 58°C for 3 min each, five times with 1 × SSC solution with 1.0% SDS at 58°C for 3 min each, and five times with 0.2 × SSC solution with 1% SDS at 58°C for 3 min each wash. The membrane was air dried and exposed on Kodak X-AR film overnight at −70°C.

DNA Sequencing

The DNA fragment excised from the band in the electrophoresed 1.5% low melting agarose was purified by Magic PCR minipreps kit (Promega, Madison, WI). The

TABLE I. Results of Human Polyomavirus Detection by Using PCR, Southern Blotting, and DNA Sequencing

Group	No.	PRC positive (No.)	Southern blot positive (No.)	JCV genotype *TW-1* (%)	JCV genotype *CY* (%)
Healthy individuals	75	11	10	50% (5/10)	50% (5/10)
Pregnant women	46	10	10	70% (7/10)	30% (3/10)
Autoimmune disease patients	28	11	10	40% (4/10)	60% 6/10)

purified DNA fragment was directly sequenced by the *f*mol DNA sequencing kit (Promega). JBR1 and JBR2 primers were used for sequencing. The detailed protocols of DNA sequencing were provided by the company (Promega).

RESULTS

Polymerase Chain Reaction (PCR)

Seventy-five urine samples from healthy individuals, 46 from pregnant women and 28 from autoimmune disease patients were examined for the presence of human polyomavirus DNA by PCR. After 40 cycles of PCR, the amplified DNA fragment was then analyzed by 1.5% agarose electrophoresis. The size of the DNA fragment generated by JBR1 and JBR2 primers was expected to be 334 base pairs. The results of PCR showed that 11 urine samples from healthy individuals, 10 from pregnant women, and 11 from autoimmune disease patients were PCR positive (Table I). To rule out possible false-negative, all samples were spiked with 10 pg of pFlag BFDV VP1 plasmid DNA [Rodgers et al., 1994] for BFDV VP1 gene amplification. The samples that showed negative in both PCR reactions were regarded as false negative and were not included in the total sample numbers.

Southern Blotting of PCR Products

The PCR products of every sample were analyzed by Southern blot to confirm that the DNA fragment shown in agarose gel was the regulatory region of human polyomaviruses and to confirm that the negative urine samples did not contain small amounts of viral DNA fragment that could not be detected by ethidium bromide staining. The results of Southern blot showed that two PCR positive samples, one from healthy individuals and the other from autoimmune disease patients, were Southern blot negative (Table I) and were regarded as nonspecific products. The PCR negative samples shown in agarose gel were still negative on Southern blot. These results indicate that the viral DNA was present in 10 urine samples from each of the three groups: 75 healthy individuals, 46 pregnant women, and 28 autoimmune disease patients (Table I).

Analysis of the Viral DNA Sequences

The DNA fragments of the Southern blot positive samples were eluted and analyzed by DNA sequencing. The results showed that JCV was the only polyomavirus present in the urine samples from these three examined groups (Table I). In addition, TW-1 and CY were the predominant strains of JCV in the examined samples, 50% of TW-1 and 50% of CY for healthy individuals, 70% of TW-1 and 30% of CY for pregnant women, and 40% of TW-1 and 60% of CY for autoimmune disease patients (Table I).

DISCUSSION

Previously, it was found that CY and TW-1 JCV and TC-1 BKV were present in the urine of pregnant women [Chang et al., 1996a] and TW-1, TW-2 and TW-3 JCV, and TC-1 and TC-2 BKV were present in the urine of autoimmune disease patients [Chang et al., 1996b]. Most of these patients are local residents in Taichung City, mid-west of Taiwan. In this report, the previous studies were extended to investigate the prevalence of human polyomaviruses in Taiwan area by examining more urine samples from individuals with different immune conditions and from different areas around the Island. JCV was found in the urine samples and the genotypes of JCV were mainly CY and TW-1.

In addition to 31 pregnant women [Chang et al., 1996a] and 20 autoimmune disease patients [Chang et al., 1996b] reported previously, we examined 200 urine samples collected Island-wide for the study of the prevalence of human polyomaviruses in Taiwan. It was found that 13.3% of healthy (immunocompetent) individuals were JCV positive, but BKV was not found in this group (Table II). About 26.0% of pregnant (transient immunocompromised) women were JCV positive and about 4% were BKV positive. The prolonged immunosuppressed patients with autoimmune disease had a higher positive rate of the presence of JCV (37.5%) and BKV (6.2%) in urine. About 24% of the 200 urine samples were JCV positive and 3% were BKV positive. It was found that 42% and 52% of JCV were CY and TW-1 genotypes respectively. Apparently, CY and TW-1 JCV are predominantly found in Taiwan.

Human polyomaviruses may persist asymptomatically and latently in renal tissue [Chester et al., 1983; Walker and Frisque, 1986]. During immunocompromised conditions, the viruses can be reactivated and be shed in urine [Apperley et al., 1987; Arthur et al., 1989; Markowitz et al., 1991]. The results are consistent with these findings. The viruses shed in the urine of immunocompetent (healthy) individuals are relatively low (13.3%). When immune status declines, the viruses are excreted increasingly in urine, 30% (both JCV and BKV) for transient immunocompromised (pregnant) women and 42% (both JCV and BKV) for prolonged immunosuppressed (autoimmune disease) patients (Table II).

The incidence of human polyomaviruses in the urine of immunocompetent individuals in the Taiwanese population has not been reported. In this study, it was found that 13.3% of the examined immunocompetent

TABLE II. Summary of the Prevalent Genotypes of Human Polyomavirus in Taiwan Including Present and Previous Results

Virus	JCV				BKV	
Group	*CY*	*TW-1*	*TW-2*	*TW-3*	*TC-1*	*TC-2*
Healthy individuals		13.3% (10/75)			0% (0/75)	
(n = 75)	50%	50%	0%	0%	0%	0%
Pregnant women		26.0% (20/77)			3.9% (3/77)	
(n = 77)[a]	40%	60%	0%	0%	100%	0%
Autoimmune disease patients		37.5% (18/48)			6.2% (3/48)	
(n = 48)[b]	39%	44%	11%	6%	67%	33%
Total		24.0% (48/200)			3.0% (6/200)	
(n = 200)[a,b]	42%	52%	4%	2%	83%	17%

[a]Including 31 cases previously reported by Chang et al. [1996a].
[b]Including 20 cases previously reported by Chang et al. [1996b].

individuals were JCV positive but BKV was not detected. In the USA, Arthur et al. [1989] showed 7% of the urine samples from the examined healthy adults were human polyomavirus positive, determined by using PCR and slot blot analysis. Kitamura et al. [1990] found that 13.2% (5/38) of examined individuals age range 0–29 were JCV positive and 2.6% (1/38) were BKV positive, determined by using blot hybridization in Japan. The results from these reports may indicate that the excretion rate of human polyomaviruses in the urine of immunocompetent individuals is similar regardless of geography.

The overall results (Table II) also show that JCV was present in 24% (48/200) of the urine samples but only 3% (6/200) for BKV. These results suggest that the incidence of JCV infection is higher than that of BKV in Taiwan. To compare with other reports from different countries, Kitamura et al. [1990] showed that about 29% (35/120) of the urine samples from individuals of various ages in Tokyo and Chiba of Japan were JCV positive and 4% (5/120) were BKV positive. These findings are similar to our results. In contrast, Markowitz et al. [1991] found that 7% (5/76) of urine samples in pregnant women from Denver, CO, were JCV positive and 15% (18/117) were BKV positive and Sundsfjord et al. [1994] showed that 16% (13/82) and 24% (20/82) of urine samples of HIV-infected patients from Bergen, Norway and Copenhagen, Denmark were JCV and BKV positive respectively. This epidemiological investigation indicates that JCV has a higher prevalence than BKV in Asian countries in contrast to Western countries.

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